STUDY OFANTIMICROBIAL PHOTODYNAMIC THERAPY FOR SELECTED FOOD-BORNE PATHOGENS

Synopsis

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INTRODUCTION

Photodynamic therapy (PDT) is an extremely important research area and has been investigated for decades for lesion treatment because of its non-invasiveness, spatiotemporal selectivity, lower side-effects and immune activation ability. It is a promising treatment modality in several medical fields, including oncology, immunology, urology, dermatology, ophthalmology, cardiology, pneumology and dentistry.

Photodynamic therapy (PDT) involves the administration of a non-toxic drug or dye known as a photosensitizer (PS) either systemically, locally or specifically to patients bearing a lesion. After an incubation period this specific lesion is then usually illuminated with a long wavelength of red visible light (620–690 nm), which, in the presence of oxygen, leads to the generation of cytotoxic species and consequently to cell death and tissue devastation. (RobertsonC. A*et. al*., 2009)

It has been used to treat various types of cancers such as malignant neoplasia, especially spread over head and neck region. Systemic rheumatoid arthritis, fungal and bacterial infections are the new fields where PDT treatment is being investigated. (Usacheva, M. N *et. al.,* 2001). It mainly consists of three components:

1. A photosensitizer
2. Light source
3. Singlet oxygen

Basically, it involves an administration of tumor-localising photosensitizer which is then illuminated with light of specific wavelength so as to activate it. The excited PS then transfers its energy to molecular oxygen, thus generating cytotoxic reactive oxygen species (ROS), such as singlet oxygen (O_2) that can oxidize key cellular macromolecules leading to tumor cell ablations.(Lucky S.S. *et. al.,* 2015).

PHOTODYNAMIC ANTIMICROBIAL CHEMOTHERAPY (PACT):

The conventional methods to combat antimicrobial resistance includes; phenotypic methods (manually by agar-dilution, gradient test, disc-diffusion and broth micro dilution) and molecular based methods such as PCR-based methods, isothermal amplification methods and DNA microarrays. However, these methods are tedious, time consuming and usually expensive thus, increasing the healthcare costs.

(Kaprou, G *et.al.*, 2021). Hence there is need to find an alternative method.

The PACT technology has evolved to compete with or replace antibiotics, reducing the burden of antibiotic resistance. (Anas A. *et. al.*, 2021*).* The basic ideology behind this therapy is that the energy absorbed via intracellular photosensitization is transferred to the oxygen molecule to cause severe damage to the oxidative reaction pathways in the plasma membrane and to the genetic materials of the microbial cells, without exerting any toxic effects on the host cells themselves. (Castano, A.P *et.al.*, 2004).

Antimicrobial treatment of food with APDT is being explored considering that the latest methods used for bacterial control in food are not always sufficiently efficient and entirely risk-free for humans and the environment. Non-thermal technologies can alter the structure of proteins and polysaccharides, causing changes in the texture, physical appearance, and functionality of the treated food. APDT has been proposed as a method of inactivation of microorganisms associated with food-borne illnesses; moreover, it is economical and environmentally friendly owing to the fact that it involves the use of non-toxic elements and induces complete destruction of the target microorganisms. (Rosa, L. P., & da Silva, F. C. 2014).

FOOD BORNE PATHOGENS:

Foodborne illness occurs when a pathogen is ingested with food and establishes itself (and usually multiplies) in the human host, or when a toxigenic pathogen establishes itself in a food product and produces a toxin, which is then ingested by the human host. Thus, foodborne illness is generally classified into:

- foodborne infection and
- foodborne intoxication.

In foodborne infections, since an incubation period is usually involved, the time from ingestion until symptoms occur is much longer than that of foodborne intoxications (BintsisT., 2017).

Humans have broader nutritional requirements than most of the microorganisms. Human diet, therefore includes a wide variety of substances from many sources. Our food thus, serves as an excellent media for microbial cultures because it is not deficient in nutrients required by microorganisms for their growth. Foods are subject to natural contamination by many different kinds

of microorganisms including pathogens. Metabolic activities of microorganisms alter the condition of food resulting in spoilage. Microbial metabolic activities change the flavour and texture of the food. Some common bacteria and fungi causing foodborne illnesses reported in the literature are shown in Table1 and Table2 respectively.

Table.1: Some common bacteria causing food borne illnesses

Food-borne disease caused by bacteria	References
Salmonella enteritidis, S. typhimurium, Staphylococcus aureus etc	Miraclin AT *et. al*., (2018)
Campylobacter sps.	Adak *et. al*., (2002)
E.coli	Adak *et. al*., (2002)
Clostridium perfringens	Adak *et. al*., (2002)
Listeria monocytogenes	Miraclin AT *et. al*., (2018)
Streptococci	Kadariya J *et.al*., (2014)
Brevibacillus borstelensis	Fangio, M. *et. al*., (2010)

Table.2: Some common fungi causing food borne illnesses

Food-borne disease caused by fungi	References
Candida albicans, C.krusei, C.lusitaniae, C.famata, C.colliculosa, C. parapsilosis, C. tropicalis etc	Rajkowska K *et. al*., (2018)
Aspergillus fumigatus	Vermorel-Faure *et.al.*, (1993)
Fusarium moniliforme	Karam, A., *et. al.*, (2005)
Rhizopus sp.	Martinello, *et. al.*, (2012)
Penicillium chrysogenum	Angelini, P *et. al., (*2006)

NATURAL PHOTOSENSITIZERS:

Plant extracts enriched with chlorophyll exert encouraging photodynamic effects due to the high quantum yield of singlet oxygen ($^1O_2$) since they show high absorption under visible light. Natural PSs are extracted from plants, fungi, and bacteria. Both natural compounds and extracts have been used as PSs for Antimicrobial Photodynamic Therapy.(Polat Ece &Kang K., 2021). Some natural photosensitizers and others used in combination that have been in the literature for APDT are shown in Table 3 and Table 4 respectively.

Table.3: Natural compounds photosensitizers used for APDT (Polat, E,& Kang,K,2021)

Chemicals constituents	**Name**	**Target organisms**	**Aim**	**Plants/ natural source**
Curcuminoids	Curcumin	*L. monocytogenes, Salmonella sp.*	Poultry	Water soluble photosensitizer curcumin
		L. monocytogenes	Food-related disease	Edible photosensitizer curcumin
		P. gingivalis, *A.actinomycetemcomitans*	Periodontitis	*Curcuma longa*. L
		V. parahaemolyticus	Seafood pathogens	Curcumin
		S. pyogenes	Pharyngotonsillitis	Curcumin formulation
		E. coli	Fresh-cut food	Curcumin
		Pseudomonas sp.	Inactivation of spoilage organism	Curcumin
		E. coli	Storage quality of fresh-cut apples	Curcumin
		P. aeruginosa	Biofilm inhibition	Curcumin
		Staphylococcus sp.	Rat model of wound healing	Curcumin gel
		A. baumannii	Multidrug resistance	Curcumin
		S. aureus	Multidrug resistance	Curcumin
		E. coli	Shelf life of fruits	Curcumin
		E. faecalis, C. albicans	Endodontic treatment	Curcumin
		Mosquito Larvae	Mosquito larva control	Curcumin
Alkaloids	Pterin-6-carboxylic acid	*K. pneumonia*	Multidrug resistance	*Baphia nitida*
Perylenequinones	Hypericin	*C. acnes*	Acne vulgaris	*Hypericum perforatum*
		E. coli	Food-borne pathogens	*Hypericum perforatum*
		P. aeruginosa	Pathogen control in a worm model	*Hypericum perforatum*
	Hypocrellin	*C. albicans*	Antifungal effect	*Hypocrella bambuase*
	Parietin	*S. aureus, S. epidermidis, E. coli, P. aeruginosa*	Local infections	*Xanthoria parietina* (L)
Anthraquinones	Anthraquinone	*L. amazonensis*	Cutaneous leishmaniasis	*Cassia sp.*
	Aloe emodin	*S. aureus, E. coli*	Food-related diseases	Aloe latex

		A. baumannii	Multidrug resistant	Aloe latex
		C. albicans	Drug-resistant	Aloe latex
Flavins	Riboflavin	*L. monocytogenes*	Listeriosis	*Bacillus subtilis*

Table.4: Photosensitizers from other sources used for APDT (Polat E, & Kang. K, 2021)

Chemical constituents	**Name**	**Target organisms**	**Aim**	**Other sources**
Chlorin-type compounds	Chlorophyllin sodium salt	*F. oxysporum, E. coli, T. aestivum*	Microbial control of contaminated wheat sprouts and seeds	Mixture of sodium and copper salts.
		R. fascians, E.amylovora, X. axonopodis	Plant pathogens and diseases	Anionic sodium magnesium chlorophyllin and Polyaspartic acid sodium salt (PA)
Porphyrin precursor	5-aminolevulinic acid	*C. parapsilosis*	Trichosporonosis	ALA synthetase

REVIEW OF LITERATURE

Some of the recent studies reported on Photodynamic therapy (PDT) and Photodynamic antimicrobial chemotherapy (APDT) are as follows:

XieJ. *et. al.*, (2021) recently reported that, various photosensitizer formulations and therapy strategies have been developed to overcome drawbacks of PDT. Significantly, the introduction of nanomaterials in PDT, as carriers or photosensitizers, may overcome the drawbacks of traditional photosensitizers. Based on this, nanocomposites excited by various light sources are applied in the PDT of deep-seated tumors.Modulation of cell death pathways with co-delivered reagents promotes PDT induced tumor cell death. Relief of tumor resistance to PDT with combined therapy strategies further promotes tumor inhibition. Also, the optimization of photosensitizer formulations and therapy procedures reduces pain in PDT.

Anas *et. al.*, (2021) studied about Photodynamic therapy (PDT) and photodynamic antimicrobial chemotherapy (PACT) combine light and photosensitizers to treat cancers and microbial infections, respectively. In PACT, the excitation of a photosensitizer drug with appropriate light generates reactive oxygen species (ROS) that kill pathogens in the proximity of the drug. PACT has considerably advanced with new light sources, biocompatible photosensitizers, bioconjugate

methods, and efficient ROS production. The PACT technology has evolved to compete with or replace antibiotics, reducing the burden of antibiotic resistance.

Niculescu AG *et. al*., (2021) investigated the interaction between light, molecular oxygen, and various photosensitizers, photodynamic therapy represents a non-invasive, non-toxic, repeatable procedure for tumor treatment, wound healing, and pathogens inactivation. However, classic photosensitizing compounds impose limitations on their clinical applications. Aiming to overcome these drawbacks, nanotechnology came as a solution for improving targeting efficiency, release control, and solubility of traditional photosensitizers.

Silva *et. al*., (2021) stated that the risk of resistance development is low due to the multi-target mode of action of antimicrobial PDT. The efficiency of APDT for microbial inactivation on foods can be affected by several factors such as type and characteristics of the PS, light source, food surface geometry, environmental aspects, and microbial characteristics. Although still a concept, the application of APDT has gained rapid acceptance in many different agri-food products and associated processes, including food production, industrial processing, storage, distribution, and retail. Most of the *in-vitro* APDT studies have provided evidence of significant antimicrobial effects, including cases in which viability was reduced up to eight orders of magnitude. Strong antimicrobial performance of APDT was also reported for studies involving variable food matrices, including fruit and vegetables, meat products, and milk.

Polat E., & Kang, K (2021) stated that Antimicrobial photodynamic therapy (APDT) is constantly evolving and can minimize this antimicrobial resistance problem. Reactive oxygen species produced when nontoxic photosensitizers are exposed to light are the main functional components of APDT responsible for microbial destruction; therefore, APDT has a broad spectrum of target pathogens, such as bacteria, fungi, and viruses. Various photosensitizers, including natural extracts, compounds, and their synthetic derivatives have been investigated.

Yagnik, D *et. al*., (2018) investigated the antimicrobial capacity of apple cider vinegar (ACV) against *E. coli, S. aureus* and *C. albicans*. The minimum dilution of ACV required for growth inhibition varied for each microbial species. For *C. albicans*, a 1/2 ACV had the strongest effect, *S. aureus, a* 1/25 dilution ACV was required, whereas for *E-coli* cultures, a 1/50 ACV dilution was

required ($p < 0.05$). Monocyte co-culture with microbes alongside ACV resulted in dose dependent down regulation of inflammatory cytokines (TNFα, IL-6). Results are expressed as percentage decreases in cytokine secretion comparing ACV treated with non-ACV treated monocytes cultured with *E-coli* (TNFα, 99.2%; IL-6, 98%), *S. aureus* (TNFα, 90%; IL-6, 83%) and *C. albicans* (TNFα, 83.3%; IL-6, 90.1%) respectively. Proteomic analyses of microbes demonstrated that ACV impaired cell integrity, organelles and protein expression. ACV treatment resulted in an absence in expression of DNA starvation protein, citrate synthase, isocitrate and malate dehydrogenases in *E-coli*; chaperone protein DNAk and filamenting temperature sensitive mutant Z(ftsZ) in *S. aureus* and pyruvate kinase, 6-phosphogluconate dehydrogenase, fructose bisphosphate were among the enzymes absent in *C.albicans*cultures. The results demonstrate ACV has multiple antimicrobial potential with clinical therapeutic implications.

Phasupan Pimonpan *et. al.*, (2021) reported that the absorbed photons were used as the basis to assess the antimicrobial photodynamic efficacy of two PSs, chlorophyll in sodium magnesium salt (Chl-Mg) and chlorophyll in sodium copper salt (Chl-Cu), under blue and white light against two typical foodborne pathogens, Gram-negative Escherichia coli, and Gram-positive *Staphylococcus aureus*. The results showed that the phototoxicity of a PS was predominantly decided by the absorbed photons rather than the characteristics of light sources. Photosensitized Chl-Mg exhibited superior antimicrobial activity as compared to that of Chl-Cu. The applied treatments were found to be more effective against *S. aureus* than *E. coli*.

Abdulrahman, H *et. al.*, (2020) investigated that curcumin with 10 J/cm^2 of light reduces *P. aeruginosa* biofilm more efficiently than without light. Extracellular polymeric substances (EPS) production was also reduced by approx. 94 % with 10 J/cm^2 of light dose. CLSM images showed that the thickness of biofilms was reduced from >30 μm to <5 μm after treatment with curcumin followed by 10 J/cm^2 of light irradiation. Curcumin showed better bacteriostatic activity than bactericidal activity. Singlet oxygen is primarily responsible for photodamage and cytotoxic reactions caused by curcumin-mediated APDT. Genes involved in quorum sensing (QS) pathway was also found to be inhibited after APDT. Curcumin with 5 J/cm^2 light inhibits QS genes and on increasing light dose *i.e*10 J/cm^2, found a drastic reduction in gene expression. Curcumin mediated A-PDT inhibits

biofilm formation of *P. aeruginosa* through QS pathway by the action of singlet oxygen generation which in turn reduced EPS of the biofilm.

Gao, J., & Matthews, K. R. (2020) investigated the antimicrobial efficacy of the water-soluble photosensitizer curcumin (PSC) on liquid media and chicken skin. The water-soluble PSC showed strong absorption at 410 nm, and the light-emitting diode (LED) used to activate PSC had strong emission at 430 nm with power density of 107 W/m^2. *Listeria monocytogenes* (3 isolates) and *Salmonella* (8 isolates) were evaluated in this study. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of PSC for *L. monocytogenes* were 10 ppm and 20 ppm, respectively. For *Salmonella*, 200 ppm of PSC led to maximum 3.6 log reduction. No significant differences for antimicrobial activities among different incubation (1, 2.5, and 5 min) or light dose (6.4, 32.1, and 64.2 kJ/m^2) were observed. Compared with the water control, no significant colour change was observed on chicken skin exposed to illumination equal to or greater than 32.1 kJ/m^2. After 5-min incubation followed by 32.1 kJ/m^2 of illumination, treatment with 300 ppm of PSC resulted in 2.9 and 1.5 log CFU/cm^2 of *L. monocytogenes* and *Salmonella* on chicken skin, respectively. PSC showed equivalent or better efficacy in reducing the population of foodborne pathogens on chicken skin, as compared with a commercial antimicrobial containing 300 ppm of peracetic acid.

Liang Z *et. al.*, (2021) conducted an experiment to inactivate *Shigella flexneri* using curcumin-based photosensitization and determine the sensitivity of *S. flexneri* to the combination of epsilon (ε)-polylysine (ε-PL) and curcumin-based photosensitization for decontamination. Bacterial numbers were reduced by 4.65 log CFU/mL after incubation with 80 μm curcumin and illumination for 20 min. Photosensitization resulted in cell morphology changes and increased cell permeability and protein degradation. Oxidative stress gene (oxyR) and efflux pump genes (marA, acrA) were upregulated. Decontamination of *S. flexneri* after incubation with curcumin and ε-PL for 1 h followed by photoactivation for 60 s has been investigated. It was found that the growth of bacteria was completely inhibited. Additionally, the number of bacteria was reduced by > 6 log CFU/ml.

Lai, D. *et. al.*, (2021) experimentally showed that curcumin-β-cyclodextrin (Cur-β-CD) could effectively generate ROS upon blue-light irradiation. The plate count assay demonstrated Cur-β-CD

complex possess desirable photodynamic antibacterial effect against food-borne pathogens including *Staphylococcusaureus*, *Listeria monocytogenes* and *Escherichia coli*. The cell morphology determined by scanning electron microscope (SEM) and transmission electron microscope (TEM) showed Cur-β-CD could cause cell deformation, surface collapse and cell structure damage of the bacteria, resulting in the leakage of cytoplasmic; while agarose gel electrophoresis and SDS-PAGE further illustrated the inactivation mechanisms by Cur-β-CD involve bacterial DNA damage and protein degradation.

Lin Y *et. al.*, (2021) indicated that treatment with 30 μmol/L curcumin and 30 min of LED light exposure could reduce bacterial counts by ~1.99 ± 0.06 log (CFU/g) in fresh *T.fusiformis* upon 5 days storage. The bacterial microbiota in *T. fusiformis* during storage was also altered upon PDT treatment. PDT treatment also retained the colour, water content, hardness, tactility, and appearance of fresh *T. fusiformis.* In conclusion, this study demonstrated that curcumin-mediated PDT could be a viable and promising non-thermal technology for preserving the quality of fresh *T. fusiformis*.

Mittal, S *et. al*.,(2013) evaluated the effect of different laser wavelengths (He–Ne: 633 nm, Nd-YAG: 532 nm), power (17, 27 mW) and duration of exposure (5, 10, 15 min) in combination with the *Beta vulgaris* natural photosensitizer on the viability of *Candida albicans* causing Candidiasis disease. Although inhibition was observed in all cases, a maximum of 51.91% inhibition takes place with the combination of *Beta vulgaris* exposed to 532 nm at 27 mW for 15 min by the Agar well diffusion method.

CHALLENGES:

PDT has extensively been investigated for decades for tumor treatment because of its non-invasiveness, spatiotemporal selectivity, lower side-effects, and immune activation ability. It can be a promising treatment modality in several medical fields, including oncology, immunology, urology, dermatology, ophthalmology, cardiology, pneumology, and dentistry. Nevertheless, the clinical application of PDT is largely restricted by the drawbacks of traditional photosensitizers, limited tissue penetrability of light, inefficient induction of tumor cell death, tumor resistance to the therapy, and the severe pain induced by the therapy (XieJ. *et. al*., 2021)

Although having so many fascinating features, application of conventional PDT has been restricted

as additional therapy to only superficial tumors due to its inability to treat deep-rooted tumors because of poor penetration depth of visible light in tissues, inefficient accumulation of PS inside the tumor mass, tumor hypoxia and heterogeneity.

To establish PDT as a frontline treatment mode and to enhance the beneficial effect of PDT, innovative approaches are being discovered to eradicate the limitations in the currently used PDT model. The latest advancements of PDT have been focused on improving light source to overcome penetration depth issues, stability and targeting ability of PSs, and tumor hypoxia.(Sarbadhikary, P *et.al*., 2021)

In recent years, there has been a surge in foodborne illnesses. Traditional methods of microbiological detection and identification of pathogenic microbes are laboratory procedures which includeaqueous polymer two phase portioning, centrifugation, filtration, ultrasound along with isolation of colonies before identification. These already discovered methods have practical merits but somehow the demerits like excessive time-consumption and labour- intensive nature are major drawbacks. There has been various progress made in the field to eradicate the problem of food poisoning and other health related issues. Progress toward the instantaneous, on-line screening of food and eradication for pathogenic organisms requires a system that can automatically separate and concentrate microbes quickly from sizeable food samples without damaging the food samples.(Benoit, P. W., & Donahue, D. W. 2003).

MOTIVATION:

The attention of scientists is shifting towards an alternative method so as to eradicate the problem of contamination by foodborne-pathogens keeping in mind issues like cost-effectiveness, natural, having no side effects and also local availability.

Photodynamic therapy can be a viable option to decontaminate food or food contact surfaces. (Le, T. D.*et. al.,* 2020). Photosensitizers will absorb photons from light source and eventually inactivate the microbial growth. The aim of this study is to evaluate and compare antimicrobial photodynamic efficacy of selected natural photosensitizer individually and in various combination against food borne pathogens. Some natural photosensitizers, namely *Ocimum sanctum* (Tulsi), *Spinacia olerace*

a(Spinach) and *Beta vulgaris* (Beetroot) have not been used individually and synergistically against food borne pathogens.

OBJECTIVES

The proposed research work aims to discover out new natural photosensitizers which have no side effects, are cost effective as well as locally available to be used individually as well as in synergism to eradicate the problem of microbial contamination in food. The specific objectives have been outlined as follows:

It is proposed to study the following aspects:

1. Maintenance and revival of foodborne microbes; that include the following

- *Aspergillus fumigatus*
- *Penicillium chrysogenum*
- *Staphylococcus aureus*
- *Brevibacillus borstelensis*

2. To study the effect of different natural photosensitizers that include the following:

- *Beta vulgaris* (beetroot)
- *Ocimum sanctum* (tulsi)
- *Spinacia oleracea* (spinach)

3. To study the effect of Physical parameters to be tested for photosensitizers that include the following:

- laser intensity (I)
- exposure time (t)
- laser wavelengths (λ)
- pulsed laser beam
- concentration of PSs

4. To comparethe effect of different natural photosensitizers individually and in synergy and synthetic photosensitizer.

- Assessment of the laser treatment by Agar Well Diffusion and Paper Disc diffusion Method

MATERIALS AND METHODOLOGY

The present study will be conducted within the premises of Dayalbagh Educational Institute, Dayalbagh Agra.

- **Maintenance and revival of foodborne microbes**

1. Maintenance of microbial cultures:

Pathogenic microbes (*Aspergillus fumigatus*, *Penicillium chrysogenum*, *Staphylococcus aureus* and *Brevibacillus borstelensis)*will be inoculated in Petri-dishes containing suitable medium directly at suitable temperature. Colonies will be transferred to other Petri-dishes for purification.

2. Preparation of medium:

Media preparation will be made accordingly. Nutrient agar media and Sabouraud dextrose agar will be made to test the presence of bacteria and fungi respectively.

3. Preservation of microorganisms:

Microbial slants will be stored at 4°C in respective media and will be revived once in a month.

- **Preparation of natural and synthetic photosensitizers**

1. Preparation of photosensitizer:

Natural and synthetic photosensitizer will be chosen to test sensitization. Natural photosensitizer will be used individually and in combination to test its efficacy against the same. Aqueous extract of plants like *Ocimum sanctum* (Tulsi), *Spinacia oleracea* (Spinach) and *Beta vulgaris* (Beetroot) will be made by using mortar and pestle. These aqueous extracts will be used as natural photosensitizer individually and in synergy against selected microbial strains possessing pathogenic property. Methylene blue will be used as synthetic photosensitizer for the experiment.

- **Study of Photodynamic therapy of microbes by laser radiations**

1. Sensitization of laser:

The wavelength of laser light will be selected upon spectrophotometrically obtained data by placing the aqueous extract of plants (natural photosensitizer) and synthetic photosensitizer in spectrophotometer

- **Comparison of chemical as well as natural photosensitizers (individually and combinations)**

1. Assessment of laser treatment:

Both natural and synthetic photosensitizer will be subjected to laser radiation. The wavelength of laser treatment will be determined by spectrophotometrically obtained data. The data exhibiting highest absorption spectra will be selected as required wavelength. The assessment will be carried out by Agar well diffusion method and disc diffusion method and their results will be compared. The Petri-plates containing bacterial pathogens will be incubated at 35± 2°C for 24 hours while the Petri-plates containing fungal pathogens will be incubated at 25± 2°C for 48-72 hours.

- Sample will be treated will laser light in the presence of photosensitizers **(group L+P+)**.
- Sample will be treated only with the photosensitizers **(Group L-P+)**
- Sample will be treated only with the laser radiations **(Group L+)**
- Sample will neither be exposed to the laser light nor to the photosensitizer **(Group C)**

- **Statistical analysis:**

By the help of the technique agar well diffusion method or paper disc diffusion method, growth diameter will be calculated. In this mean, standard error, percentage inhibition and coefficient of correlation will be calculated.

A) Calculation of mean: Arithmetic mean of a group of observations is the quotient obtained by dividing the sum of all the observations by their number. Thus, arithmetic mean denoted by X or A.M.

$$X = \frac{\text{Total no. of all values}}{\text{Number of values}} = \frac{\Sigma X}{N}$$

B) Standard error: The standard derivation of a sampling distribution of a statistic is also called standard error.

$$\text{S. Error} = \frac{SD}{\sqrt{N}}$$

Here, SD is standard deviation which is calculated as:

C) Standard deviation:

$$\textbf{SD: } \sqrt{\frac{\Sigma x^2}{N}} \quad \textbf{Here } x = X - \overline{X}$$

Percentage inhibition:

The reduction in mycelia biomass only for fungi was calculated by percentage inhibition using formula:

D) % Inhibition = 100 x (C-T) / C

Where,

C = Bacterial colony diameter in control.

T = Bacterial colony diameter in various treatment.

Statistical analysis will be performed by calculating the correlation and T-test using 5% level of significance. This will further help to compare and analyze the data obtained from testing different concentration against various parameters and find out the most effective natural photosensitizer against the pathogens. It will be carried out using SPSS SOFTWARE.

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